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The rise, fall and resurrection of chemical induced resistance agents

Running title: *Chemical IR: rise, fall and resurrection*

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Abstract

Since the discovery that the plant immune system could be augmented for improved deployment against biotic stressors through the exogenous application of chemicals that lead to induced resistance (IR), many such IR-eliciting agents have been identified. Initially it was hoped that these chemical IR agents would be a benign alternative to traditional chemical biocides. However, owing to low efficacy and/or a realisation that their benefits sometimes come at the cost of growth and yield penalties, chemical IR agents fell out of favour and seldom used as crop protection products. Despite the lack of interest in agricultural use, researchers have continued to explore the efficacy and mechanisms of chemical IR. Moreover, as we move away from the approach of 'zero tolerance' toward plant pests and pathogens toward integrated pest management, chemical IR agents could have a place in the plant protection product list. In this review, we chart the rise and fall of chemical IR agents, and then explore a variety of strategies used to improve their efficacy and remediate their negative side effects.

Keywords

Induced resistance, priming, IPM, trade-offs, synergistic, biological control

1 INTRODUCTION

In recent decades, the philosophy behind the control of plant pests and pathogens has been driven by a 'zero tolerance' approach, where elimination of the causal agent is the unstated aim. As this has rarely, if ever, been achieved, the extreme selection pressure exerted on the surviving pest and pathogen populations presents obvious dangers, such as rendering genetic resistance ineffective or resulting in populations acquiring resistance to biocidal chemical agents. An alternative, however, is to take advantage of recent advances in our understanding of plant-microbe interactions and use alternative control strategies that leverage the plant immune system in a systems context, namely Integrated Pest (/crop) management.

Plants possess a sophisticated innate immune system that provides the first line of defence against attackers. This is controlled by a complex network of interconnected signalling pathways that are directly activated upon recognition of Microbe-Associated Molecular Patterns (PAMPs) and/or Damage-Associated Molecular Patterns (DAMPs). The model of plant–pathogen interactions by Jones and Dangl (2006)[1], also referred to as the 'zig-zag' model, is perhaps the most popular model of the plant innate immune system which distinguishes three forms of disease resistance. Effector-triggered immunity (ETI) – commonly known as race-specific or vertical resistance – is a qualitative form of disease resistance that relies on the presence of single resistance genes (*R*). The associated R proteins enable direct or indirect recognition of susceptibility-inducing pathogen effectors and activate a rapid immune response, which is typically associated with hypersensitive cell death. Accordingly, ETI provides high levels of protection against biotrophic pathogens.[2] However, because of its monogenic nature, ETI has a narrow range of taxonomic effectiveness and limited durability due to the evolutionary pressures on pathogens to

evolve alternative effectors, thereby avoiding recognition by R proteins.[3,4] Pattern Triggered Immunity (PTI) is a quantitative form of disease resistance, which provides high level resistance against a broad range of attackers. PTI is triggered by a multitude of conserved molecular patterns that are produced during infestation or infection by pests and diseases, respectively, which activate a range of different pathways and defence mechanisms that become active at different stages of the interaction. However, PTI is not sufficiently effective against virulent pathogens[1,5], which employ effector molecules that subvert PTI-controlling pathways, a process commonly referred to as Effector-Triggered Susceptibility (ETS).[1,6] In addition to PTI-suppressing effectors, ETS by biotrophic pathogens also involves 2nd level effectors that suppress ETI-related signalling and hypersensitive cell death-related.[1,7,8] Within the framework of the zig-zag model by Jones and Dangl (2006)[1], the residual level of resistance after ETS-mediated repression of PTI and ETI is referred to as basal resistance (BR)[1]. Since its inception, the zig-zag model has been interpreted as a co-evolutionary arm's race, during which pathogens evolved ETS to suppress PRR-dependent PTI and plants counter-evolved R-proteins to recognise effector activity and activate ETI.

Although proven exceedingly useful for the conceptual interpretation of plant innate immunity and evolution, the zig-zag model is not without limitations.[9] Foremost among them is that the model only represents plant innate immunity against biotrophic pathogens. Furthermore, while it is acceptable to portray ETI, PTI and BR as different types of resistance within an evolutionary context, they are remarkably similar from a mechanistic point. All three types of resistance share similar signalling pathways and defence mechanisms that become active during different stages of the interaction with avirulent, non-host and virulent pathogens, respectively.[10,11] These pathways and mechanisms include relatively early-acting local defences , such as the accumulation of reactive oxygen species and cell

wall reinforcements.[12–15] Also, there are later-acting defences that are controlled by *de novo* produced defence hormones, such as salicylic acid (SA), jasmonic acid (JA), ethylene (ET) and abscisic acid (ABA), [16,17] which all interact with each other to prioritise and fine tune an appropriate immune response.[18,19] Hence, from a mechanistic point of view, there is no clear partition between ETI, PTI and BR.

Although the plant innate immune system protects against the majority of potentially hostile microbes, it cannot prevent infection and damage by virulent pathogens. To minimise damage by these attackers, plants have evolved the ability to augment the level of innate immunity by forming a memory of previous pathogen encounters, resulting in a faster and/or stronger deployment of inducible plant defence mechanisms upon subsequent encounters. This so called **defence priming** results in induced resistance (IR), which is a form of phenotypic plasticity and can thus be regarded as plant acquired immunity.[20] IR is often systemically expressed and has the benefits of being durable with broad-spectrum effectiveness, while also providing protection that is stronger than BR.[21] Given the ability to augment plant resistance, many natural and synthetic IR-eliciting agents have been identified and characterised in detail. However, to date, these products are not widely employed in crop protection schemes. In this review, we assess the rise of IR agents, initially seen by some as silver bullet solutions for benign crop protection, and their subsequent fall out of favour, owing to low efficacy and/or a realisation that their benefits sometimes come at the cost of growth and yield penalties. Finally, we explore how we can use our increased understanding of host-microbe interactions to facilitate a resurrection of IR agents as tailored components of plant protection methods that are implemented in a systems context, namely within Integrated Pest Management (IPM).

2 THE RISE AND FALL OF CHEMICAL IR AGENTS

Six decades ago, Ross (1961)[22] observed that localised infection of tobacco plants with tobacco mosaic virus (TMV) leads to immunity in distal non-infected leaves. This so called systemic acquired resistance (SAR) is a form of IR and is dependent on the plant defence hormone salicylic acid (SA) and the defence regulatory protein NPR1.[23] Activation of this pathway results in direct activation and priming of a wide range of different basal defence mechanisms, including the production Pathogenesis Related (PR) proteins. The priming associated with SAR can provide long-lasting protection against a broad spectrum of (hemi-)biotrophic pathogens.[20,22–24] In subsequent studies, it became clear that there are additional IR responses, which are controlled by partially different signalling pathways. environmentally, which is triggered by root colonisation with beneficial soil microorganisms, such as plant growth-promoting rhizobacteria (PGPR), endophytic plant growth-promoting fungi (PGPF) and arbuscular mycorrhizal fungi (AMF), is under control by a signalling pathway partially different from SAR. In *Arabidopsis*, ISR is dependent on the defence regulatory protein NPR1 but operates independently of SA.[25] Instead, ISR is typically based on a priming of JA- and ET-dependent signalling pathways.[26,27] Based on prior discovery of JA as a wound-responsive defence hormone in plants,[28] JA and its methylated derivative methyl-jasmonic acid (MeJA) have often been used as chemical IR agents against herbivores and necrotrophic pathogens. [29,30] Moreover, while SAR is predominantly effective against biotrophic pathogens, ISR is more effective against necrotrophic pathogens.[31,32] Further evidence, for the existence of alternative forms of IR came from the characterisation of β-aminobutyric acid-induced resistance (BABA-IR). BABA is a non-protein amino acid that is produced in low concentrations by stressed plant tissues.[33] Perception of BABA is dependent on the IBI1 receptor gene, which encodes an aspartyl-tRNA synthetase and

controls BABA-IR against downy mildew and necrotrophic fungi.[34] Furthermore, the underlying signalling pathways of BABA-IR vary according to the challenging pathogen and can either be SA-dependent or SA-independent[35,36], providing broad-range protection against biotrophic and necrotrophic pathogens.[37] The three classic examples of SAR, ISR and BABA-IR illustrate IR is controlled by a variety of different defence signalling pathways, depending on the eliciting agent, plant species and challenging pathogen. Despite this diversity, all IR responses share the common characteristic that they augment the effectiveness of BR through either a direct up-regulation or a priming of basal defence mechanisms.[20]

To maximise the benefits of SAR, White, (1979)[38] showed that injections of SA, aspirin and benzoic acid, each elicited SAR against *tobacco mosaic virus* (TMV) in tobacco. This pioneering experiment showed that SAR can be triggered without having to infect plants with pathogens and heralded an era of research into chemical IR agents. Research throughout the 1980s and 1990s led to the development of several functional SA analogues that act as potent SAR inducers, of which the best known are 2,6-dichloroisonicotinic acid (INA) and its derivative Acibenzolar-S-methyl (ASM). INA was shown to provide high level of protection in different crops including barley, cucumber and rice.[39–41] Similarly, ASM showed high resistance-inducing efficacy in a range of different crop pathosystems.[42–45] Based on these results, Syngenta launched Actigard®/Bion® as the first commercial IR agent, which includes ASM as the active ingredient. Other IR agents, such as BABA[33,37] and Chitosan, a polymeric derivative of chitin[46], yielded similarly high levels of crop protection against economically devastating plant diseases. Accordingly, IR agents emerged as an appealing alternative to fungicides, since they show little or no direct toxicity towards the pathogen or

environment, while providing broad-spectrum protection through augmentation of durable BR.[47]

However, the initial ambition to employ chemical IR agents as main-stream crop protection products never materialised, which was largely due to undesirable non-target effects on plant growth and seed. This was first highlighted by Heil *et al*. (2000)[48], who showed that wheat plants treated with ASM had lower biomass, developed fewer shoots and produced fewer seeds compared with untreated plants and this was particularly pronounced in plants grown with a limited nitrogen supply. Although a direct up-regulation of basal defence mechanisms could achieve high levels of protection, the associated costs made these agents less attractive for commercial exploitation as crop protection products. It was argued that the deployment of IR agents is only beneficial under conditions of high disease pressure, where the associated costs are outweighed by the benefits of disease protection.[48–51] Besides being metabolically costly, IR activators could also be phytotoxic. INA and its derivatives were deemed too toxic for agricultural use.[52] Similarly, BABA was found to cause toxicity via inhibition of AspRS enzyme activity.[34] A third obstacle associated with chemical IR agents is that their efficacy can be highly variable between plant genotypes. In both cucumber[40] and soybean[53] INA efficacy varied by genotype. Efficacy may also be affected by the pathogen strain. In tomato, disease protection by BABA not only varied by host genotype but also by *Phytophthora infestans* isolate.[54] Additionally, there is compelling evidence that environmental conditions affect the outcome of chemically induced IR.[55,56] Furthermore, chemically induced IR is generally transient lasting at most weeks [57–60] which necessitates multiple applications. This complex interplay of variables affecting IR efficacy has impeded wide-spread adoption of chemical IR agents in agriculture and horticulture.

3 THE RESURRECTION OF CHEMICAL IR AGENTS

3.1 Plant defence priming

The costs associated with prolonged expression of defences, has resulted in the evolution of **priming** as a more cost-efficient strategy for IR, which allows plants to mount a faster and/or stronger BR response against attackers.[61,62] Although priming typically manifests itself as a long-term consequence of transient defence induction to biotic stress, chemical IR agents can serve as suitable priming stimuli when applied in relatively low doses.[50] In some instances, plants receiving such treatments have been shown to display minimal defence induction before pathogen encounter, although their effectiveness tends to be lower than chemically induced IR mediated by direct up-regulation of defences.[63,64] Furthermore, IR via priming is still associated with a reduction in plant growth and seed set, albeit minor, which can make it unfavourable in stress-free conditions.[20,62,65] However, these costs are outweighed by the benefits of protection under stressful conditions.[62,63,66] Given the significance of priming for plants in their natural environment, it has strong potential to be developed into an energetically (and environmentally) benign plant protection strategy. To this end, it is necessary to ascertain how a given IR chemical behaves - for instance, at what concentrations do IR agents switch from priming activity to a more costly direct induction of basal defences? Regardless of the nature of the priming stimuli, Martinez-Medina *et al*. (2016)[62] proposed a set of sequential criteria that must be satisfied, namely 1) a memory of the priming stimulus with a low fitness cost, and 2) a stress trigger that induces a faster and/or stronger defence response resulting in improved disease protection. Indeed, since the potential of priming was highlighted by Conrath *et al*. (2006)[61], the capacities of priming chemicals, both natural and synthetic, have been documented in a variety of plant pathosystems.[67] Although it is now commonly acknowledged that the use of priming

chemicals in agriculture is reduced by their limited efficacy and variable performance, optimising their potential as components of IPM is becoming appealing.[68–70]

3.2 Integrating chemical IR agents in to IPM

IPM is a strategy for combating plant pests and diseases, using all available environmentally benign methods whilst minimising the applications of chemical pesticides, to keep them below the economic injury level (EIL) threshold. Chemical IR agents fit well into IPM as they can be a replacement for a conventional pesticide or they could be a means of reducing their dosage. Moreover, other components commonly used in IPM could be used as means to improve some of the problems associated with chemical IR agents and thus make them more efficacious. However, IPM is applied to multiple crops with multiple pathogens, some of which are coincidental in time and/or space. Therefore, it is important to understand the principles whereby IPM components are combined and how these will impact different host-pathosystems. In the remainder of this review, we explore various approaches to improve the efficacy of chemical IR agents (Table 1), and discuss how these can be included within IPM strategies.

214 **Table 1: Strategies used to improve the efficacy of chemical IR agents.**

Strategy	Agent(s)	Pathosystem		Effect	Ref
Combining biocontrol and chemical IR	MeJA – *T. harzianum*	wheat	*Bipolaris sorokiniana*	Reduced symptoms. Combination more effective than either treatment alone. Increased biomass	71
	MeJA – SA – *T. harzianum*	tomato	*Fusarium oxysporum*	Synergistic induction of defences. Increased biomass	72
	ASM – *T. harzianum*	faba bean	*Botrytis fabae*	Combination improved efficacy	73
			Botrytis cinerea	Combination gave complete protection	
	ASM – *A. pullulans*	kiwifruit	*Pseudomonas syringae*	Combination improved efficacy	74
Combining chemical IR agents	ASM – BABA – *cis*-jasmone	barley	*Ramularia collo-cygni*	Improved efficacy. Reduced toxicity	75
	ASM – BABA	grapevine	*Plasmopara viticola*	Additive protective effective	76
Combining chemical IR agents and fungicides	BABA – Mancozeb	potato	*Phytophthora infestans*	Synergistically increased its fungicide efficacy.	77
		tomato			
		cucumber	*Pseudoperonospora cubensis*		
	BABA – Fluazinam	potato	*Phytophthora infestans*	Full fungicide activity achieved with a 20–25% lower dose	78
	ASM – Mancozeb	chickpea	*Didymella rabiei*	ASM application frequency reduced. Improved grain yields.	79
	BABA – Fosetyl-Al	grapevine	*Plasmopara viticola*	Additive protective effective with half recommended fungicide dose	76
	BABA – N-(Trichloromethylthio) Phthalimide				
Rationally designed IR agents **Ionic Pairing**	[$BABA^-$] [$Cholinium^+$]	tobacco	tobacco mosaic virus	Reduced phytotoxicity	80
	[$ASMCOO^-$] [$Cholinium^+$]			Reduced phytotoxicity. Improved disease resistance	
	[INA^-] [$Cholinium^+$]			Improved disease resistance	
Rationally designed IR agents **Structural analogues**	L1-3a and L1-4a novel benzotriazole	cucumber	*Botrytis cinerea*	Efficacy comparable to ASM	81
		tomato	*Phytophthora infestans*		
	RBH new IBI1 ligand	*Arabidopsis*	*Hyaloperonospora arabidopsidis*	Resistance to both biotrophic and necrotrophic pathogens without growth retardation	82
			Plectosphaerella. cucumerina		
		tomato	*Botrytis cinerea*		
Multi-action IR agents	Strobilurins (Broad-spectrum fungicides)	wheat		Improved plant growth	83
		tobacco	*Pseudomonas syringae*	The strobilurin pyraclostrobin conferred IR in SAR	84
			tobacco mosaic virus		

				deficient *NahG* transgenic tobacco	
	1-isothiocyanato-4-methylsulfinylbutane	*Arabidopsis*	*Hyaloperonospora arabidopsidis* *Plectosphaerella cucumerina* *Pseudomonas syringae*	Induced resistance Direct antimicrobial action	85
Transgenerational IR	Aescin	*Arabidopsis*	*Pseudomonas syringae*	A member of the antimicrobial saponins. Induced resistance	86
	BABA	*Arabidopsis*	*Hyaloperonospora arabidopsidis* *Pseudomonas syringae*	Progeny became more responsive to BABA priming	87
	BABA INA	common bean	*Pseudomonas syringae*	Enhanced transgenerational resistance	88
	MeJA	*Arabidopsis*	Caterpillar	Increased resistance in progeny to caterpillar herbivory	89

3.3 Combining biocontrol and chemical IR

One approach to increase the protection levels of chemical IR agents is to combine them with other agents. Several studies have shown that chemical IR agents and biological control agent (BCAs) in combination results in improved disease control. BCAs are naturally occurring communities antagonistic to specific plant pests and pathogens that have minimal non-target effects [90] and a common component of IPM. The most investigated BCAs in this regard are the *Trichoderma spp*, which grow chemotropically toward the roots of many crop species. In the roots, they produce various metabolites that promote plant growth through enhanced nutrient availability. Furthermore, the *Trichoderma* also induce plant defence pathways and ultimately inhibit plant pathogens[91]. In bread wheat plants (*Triticum aestivum* L.) receiving combined MeJA and *Trichoderma harzianum* UBSTH-501, spot blotch (*Bipolaris sorokiniana*) symptoms were reduced significantly in comparison to plants receiving either treatment alone. The efficacy of this combined treatment corresponded with enhanced production of the plant development and growth promoter,

indole acetic acid in the plant rhizosphere.[71] In another study, MeJA, SA and *T. harzianum* treatments individually gave a similar level of protection against *Fusarium oxysporum* wilt disease in tomato. However, their combination resulted in a synergistic induction of tomato antioxidant defences against *F. oxysporum*.[72] Similarly, combining *T. harzianum* and ASM was significantly better at controlling *Botrytis fabae* disease severity in faba bean plants than either treatment alone.[73] Whilst in most cases the complementary protection conferred by BCAs and chemical elicitor combinations is not complete, in some cases it has been possible to give a high level of protection. A combination of *T. harzianum* and ASM was shown to give complete protection in faba bean plants against *Botrytis cinerea* infection.[73] Other BCAs have also shown to complement chemical IR agents. For instance, the saprophytic yeast-like fungus *Aureobasidium pullulans* CG163 in combination with ASM showed significantly reduced leaf spot incidence compared to untreated plants. The CG163+ASM combination treatment was more effective than either treatment alone. Furthermore, in plants receiving both treatments there was significant upregulation in expression of the defence related genes *PR1*, Class IV chitinase and β-1,3-glucosidase. This change in gene expression correlated positively with treatment efficacy and expression was highest in plants receiving the combined CG163+ASM.[74]

BCA-chemical IR agent combinations, in addition to improving the protective efficacy, have also been shown to improve growth. In bread wheat plants, combined MeJA and *T. harzianum* treatment resulted in significantly higher biomass, both in the presence and absence of *B. sorokiniana* infection.[71] In tomato, combining MeJA or SA with *T. harzianum* improved the protection against *F. oxysporum* disease incidence more than treatment with SA or MeJA alone. Furthermore, due the improved protection, biomass was also significantly higher in plants receiving the combined treatment. [72]

3.4 The compatibility of chemical IR agents with biocontrol organisms

Given the broad-spectrum effectiveness of non-host immunity, chemical treatments intended to trigger IR responses against plant antagonists could also cause deleterious effects on plant mutualists, and so the combinations of chemical IR agents and BCAs in IPM needs careful selection. Examining the effects of IR establishment by ASM application on soybean-rhizobia and soybean-AMF mutualisms, *in vitro* the chemical had no direct effect on the growth of the rhizobia *Bradyrhizobium japonicum* and only a slight inhibition at very high doses on the AMF *Glomus mosseae*. However, both seed and foliar spray application caused increased IR biochemical markers, reduced *B. japonicum* soybean symbiosis efficiency and reduced *G. mosseae* mycorrhization in soybean.[92] A similar finding was also reported by de Román *et al*. (2011)[93] who found foliar treatment of soybean with ASM led to a significant, but moderate, defence response in the plant roots which transiently decreased AMF colonisation. This defence induction was not associated with an allocation cost, and so the negative effects on AMF colonisation were likely due to defence induction rather than changes in resource allocation. Nevertheless, chemical IR treatments do not always impact plant mutualists negatively and it seems that with some chemicals, certain doses and appropriate application methods, they can be used together without disadvantage to plant mutualists. In sunflower, the effects of ASM and BABA on the downy mildew *Plasmopara helianthi* and the AMF *G. mosseae* differed by application method. When applied as a soil drench, the chemicals gave a 50-55% protection against the downy mildew - while ASM application decreased *G. mosseae* colonisation, BABA application did not. When applied as a foliar spray, protection increased to 80% and neither chemical impacted *G. mosseae* colonisation. *In vitro*, ASM had an inhibitory effect on *G. mosseae* germination, however BABA promoted germination.[94] In other studies, the negative effects

of chemical IR agents on plant mutualists was shown to be dose-dependent. In soybean, SA root application had no impact at lower doses typically used to induce resistance and only had a negative impact at very high doses.[95] Similarly, MeJA root application to cucumber could negatively or positively effect mycorrhizal colonisation, with higher doses reducing growth and lower doses promoting it.[96]

3.5 Combining chemical IR agents

Combining different chemical IR agents has also shown promise under field conditions. In barley, Walters *et al*. (2011)[75] found improved control of powdery mildew using ASM, BABA and JA combined treatments. Given the growth costs associated with higher and more protective doses in many chemical IR agents, using low doses of multiple agents for additive or synergistic IR effects with minimal growth costs is a potential means of improving their efficacy. In one study, Reuveni *et al*. (2001)[76] established that BABA – ASM mix applied at half the recommended dose had an additive effect, effectively controlling *Plasmopara viticola* in grapevines. Despite this early promise, the strategy of combined chemical IR agents has received little further attention.

3.6 Combining chemical IR agents and fungicides

Similarly, results from chemical IR agent – biocide combinations show a complementary potential in which any deleterious effects of both protection products can be reduced. An application of a mixture of BABA and the fungicide mancozeb was significantly more effective at controlling potato late blight (*P. infestans*) as well as tomato and cucumber mildew (*Pseudoperonospora cubensis*) than either BABA or mancozeb alone. The inclusion of BABA in the mancozeb fungicide synergistically increased its efficacy in plants with 5:1 BABA: mancozeb showing the highest synergy factor. Application of the BABA and

mancozeb mixture did not have a synergistic interaction in controlling the pathogens *in vitro*, thus demonstrating BABA-induced resistance enhanced mancozeb fungicide efficacy with lower doses required to control disease.[77] In potato, a combination of BABA and the fungicide Fluazinam resulted in a synergistic action against late blight. Furthermore, full Fluazinam activity was achieved with a 20–25% lower dose under field conditions.[78] Likewise, ASM efficacy improved in combination with mancozeb. In chickpea plants, repeated ASM application protected against chickpea blight (*Didymella rabiei*) but also resulted in yield penalties. Instead, using a ASM – mancozeb mix, with reduced application frequency, grain yields were better than those achieved with ASM or mancozeb applications alone.[79]

3.7 Dual action IR agents

Besides the combination of chemical IR agents with fungicides, another strategy employed to improve their performance has been identifying compounds combining biocidal and IR activity. One group of chemicals with such dual modes of action are the strobilurins, introduced in the 1990s as broad-spectrum fungicides. It became apparent they also improved plant health and yield in the absence of disease pressure and prime plant defences. In *NahG* transgenic tobacco deficient in SAR, the strobilurin Pyraclostrobin enhanced resistance to *Pseudomonas syringae* and TMV by priming *PR-1* gene activation.[83,84] In an effort to find dual action compounds Schillheim *et al*. (2018)[85] developed a high-throughput assay to screen cultured parsley for compounds that prime the secretion of antimicrobial phytoalexins and found 1-isothiocyanato-4-methylsulfinylbutane (SFN). In *Arabidopsis*, this compound primed *WRKY6* gene expression and reduced susceptibility to *Hyaloperonospora arabidopsidis*. Additionally, SFN showed broad antimicrobial action, directly inhibiting the growth of the oomycete *H*. *arabidopsidis*,

the fungus *Plectosphaerella cucumerina* and the bacterium *P. syringae*. Also turning to natural plant antimicrobials to find dual action molecules, Trdá *et al*. (2019)[86] compared the antifungal activities of several members of the Saponins, a group of compounds found in several plant species and considered antimicrobial. Among the saponins tested, aescin showed the strongest antifungal activity. In terms of plant defence induction, aescin showed strong defence induction in Rapeseed against *Leptosphaeria maculans* and in *Arabidopsis* against *P. syringae*.

3.8 Rationally designed chemical IR agents

In other approaches, researchers used rational design to develop a range of new or modified IR molecules. To improve efficacy and reduce phytotoxicity, Kukawka *et al*. (2018)[80] took the approach of ionic pairing by combining various IR agents with the cholinium cation to form ionic liquids (ILs). BABA, ASM and INA ionically bonded to cholinium – an essential nutrient in the cells of many organisms and which is non-toxic and biodegradable[97] – were tested on the tobacco-TMV pathosystem. ASM and INA, paired with cholinium, had improved disease resistance efficacy. BABA disease efficacy decreased slightly; however, its phytotoxicity, along with that of ASM, drastically reduced.

Since the development of INA and ASM, improvements in large-scale chemical screens and computer aided drug design have enabled the screening of vast numbers of chemicals for IR properties at a relatively low cost. Chang *et al*. (2017)[81] virtually screened the Maybridge database, a collection of over 53,000 organic compounds, using the chemical structures of ASM, MeSA and SA to identify three benzotriazole lead compounds. From one of these (L1), which had a 3D structure similar to ASM, two derivatives (3a and 4a) were potent SAR

activators. Both L1-3a and 4a gave high protection in a several pathosystems including cucumber- *B. cinerea* and tomato- *P. infestans*.

In addition to screening for structural analogues of known IR molecules, using knowledge of IR receptor structure has been another approach taken to find novel IR ligands. Buswell *et al*. (2018)[82], in an attempt to find BABA analogues that induce resistance without stunting plant growth, started with the structure of the BABA receptor IBI1 and through site-directed mutagenesis, found that an (I)-aspartic acid-binding domain was critical for BABA perception. Using ligand-interaction modelling of the binding domain they screened a library of β-amino acids and identified seven resistance-inducing compounds, of which *(R)*-β-homoserine (RBH) had the strongest activity. RBH, like BABA conferred resistance to both biotrophic and necrotrophic pathogens in taxonomically unrelated plant species but without the growth retardation associated with BABA.

3.9 Selecting optimal pathosystems for priming

Understanding species, cultivar and pathogen-dependent responses to chemical IR treatments is crucial to selecting pathosystem appropriate treatments. Chemical IR agent efficacy in some instances is known to be cultivar dependent. In several cultivars of spring barley induced resistance to *Rhynchosporium commune (*formerly *Rhynchosporium. secalis)* by combined BABA, ASM and MeJA treatment resulted in infection levels that ranged from high to non-existent.[98] In other studies, chemical IR treatment efficacy was shown to be influenced by cultivar resistance levels. In tobacco infected with *Peronospora hyoscyami f.sp. tabacina*, ASM provided effective control in partially resistant cultivars, but not susceptible cultivars.[99] Likewise, in cucumber INA efficacy against *Sphaerotheca fuligenea* infection was best in partially resistant cultivars.[40] In contrast, both ASM and INA

efficacy against *Sclerotinia sclerotiorum* in soybean was superior in susceptible cultivars.[53] Similarly, the efficacy of chemical IR agents can also depend on the identity of the attacking pathogen. In tomato, ABA application lead to antagonistic cross-talk between the ABA- and SA-responsive defense pathways, resulting in increased susceptibility to *B. cinerea*[100], while in *Arabidopsis* pre-treatment with SA caused cross-talk between the SA and JA-dependent defense, causing increased susceptibility to *Alternaria brassicicola*.[101] In barley, saccharin, a derivative of probenazole, gave high levels of protection against the biotrophic fungi *Blumeria graminis*[102] and the hemibiotrophic fungus *R. commune*[103], while in *Arabidopsis* it protected against infection by hemibiotrophic *P. syringae* DC3000.[104] However, saccharin of *Arabidopsis* also caused increased susceptibility to the necrotrophic pathogens *B. cinerea* and *Pectobacterium carotovorum*, presumably due to antagonistic signalling cross-talk. Indeed, saccharin treatment of *Arabidopsis* resulted in the upregulation of SA-responsive genes and the simultaneous downregulation of JA-responsive genes.[104] In addition to some chemical IR agents resulting in increased susceptibility to some pathogens, mixtures of chemical IR agents may lead to undesirable outcomes due to the complex cross-talk between plant defence pathways. However, apart from considerable evidence that SA and JA dependent defence pathways are antagonistic [105], there is evidence of the simultaneous expression of SA- and JA-mediated defences.[106–109] Mur *et al*., (2006)[110] found that co-treatment of tobacco and Arabidopsis with relatively low concentrations of SA and JA resulted in transient synergistic effects on the expression of SA- and JA-dependent defence genes, while higher concentrations of these hormones resulted in antagonism.[110] In wheat, simultaneous application of MeJA and *T. harzianum* followed by challenge with *B. sorokiniana* resulted in the induction of both JA- and SA-dependent defence signalling. *T. harzianum*-treated plants showed increased SA levels, enhanced accumulation of total free

phenolics and increased activities of defence-related enzymes, but addition of MeJA to *T. harzianum* treatment did not affect SA induction.[71] By contrast, in freesia inflorescences, MeJA significantly reduced *B. cinerea* disease severity but the addition of ASM to MeJA significantly reduced its efficacy.[111] Similarly, in barley, combined treatment of ASM, BABA, and cis-jasmone activated SAR, while suppressing the JA signalling pathway.[75] Treatment resulted in an up-regulation of the SAR marker *PR1-b* and a substantial down regulation of the *LOX2* gene involved in JA biosynthesis. Furthermore, plants receiving this combination treatment became resistant to powdery mildew, which is effectively controlled by SA-dependent defences. At the same time, plants became more susceptible to the hemi-necrotrophic leaf spot pathogen *Ramularia collo-cygni*, which is controlled by JA-dependent defences.[75]

3.10 Transgenerational IR

Since the first systematic studies by Ross in the 1960s, IR has been portrayed as a long-lasting resistance response. Only recently, this aspect of IR has gained renewed attention in the context of epigenetic regulation. Seeds or seedlings treated with chemical IR agents develop a long-lasting priming that can be maintained for several weeks [58,112]. Furthermore, following sporadic early reports that progeny from biotic stress-exposed plants, such as tobacco by TMV[113] and wild radish by caterpillars[114], there is now solid evidence from independent studies that priming can be transmitted epigenetically to following generations. Slaughter *et al*. (2012)[87] reported that progeny of BABA-treated *Arabidopsis* displayed enhanced resistance to *H. arabidopsidis* and *P. syringae*, which was associated with increased responsiveness to priming treatment by BABA ('primed to be primed').[87] Walters and Peterson (2012)[115] showed that barley from acibenzolar-S-methyl- and saccharin-treated parents exhibited enhanced resistance to infection by *R. commune*.

Furthermore, treatment of common bean with both BABA and INA resulted in transgenerational IR against *P. syringae*[88], while MeJA-treated *Arabidopsis* was found to produce progeny that is primed for JA-dependent defences against herbivory.[89] A suite of recent Arabidopsis-based studies have shown that transgenerational IR relies on a complex interplay of DNA (de)methylation pathways in the plant.[20,116–119] Despite these promising new insights, the potential of IR agents to exploit transgenerational IR in the field has received limited attention. The main obstacles come from the relative weakness of transgenerational IR, as well as costs arising from increased susceptibly to other (a)biotic stresses.[116,120] A potentially more promising strategy for the exploitation of transgenerational IR comes from direct manipulation of the epigenetic makeup of the plant. Furci *et al*. (2019)[119] identified selected hypo-methylated regions of DNA in the Arabidopsis genome, which provided near complete levels of primed resistance against downy mildew and that remained stable over at least 8 generations of inbreeding.

3.11 Chemical IR in practical crop protection

With the continuing expansion of our understanding of the mechanistic basis of IR, the characterisation of the action of many chemical IR agents in many pathosystems and the availability of more effective agents, it is reasonable to hope that these agents have the potential to become widely used crop protection products. In the field, prediction of the actions of applied chemical IR agents is difficult as this is a relatively uncontrolled environment where many abiotic and biotic stresses will trigger plant responses that can lead to complex interactions with the agents[51,121,122] and so their use must be carefully targeted. However, in more controlled environments such as glasshouses or highly controlled vertical farming chambers, their potential is high. Under such controlled conditions, it should be possible to combine IPM measures that include chemical IR agents

in a way that has more predictable outcomes. Also, under these controlled environments, there is a scope for formulating bespoke treatments that are highly targeted to the biotic stress vulnerabilities of the system. Furthermore, for organic growers that desire natural means of protecting produce, the exploitation of IR agents can fulfil such requirements. Indeed, interest in 'natural' protection products is growing. The global plant Biostimulants (a term used for commercial products that are marketed as stimulants of natural plant growth and/or protection) market is forecast to reach USD4.5 billion by 2027 and have an annual growth rate of 11.2% during the period 2020-2027.[123] In order to provide improved products to this growing market, it is necessary to increase the translation of the growing mechanistic knowledge of IR, in to applied research that incorporates chemical IR in to IPM.

4 CONCLUSION

Chemical IR agents that lack biocidal action but instead augment plant resistance to invaders may be a viable option in the tool kit for plant pest and pathogen control. These chemical IR agents, initially billed as cost free potential alternatives to conventional pesticides, have not been widely used in agriculture, limited by their insufficient efficacy compared with conventional biocides, variable efficacy and yield penalties. Although, achieving levels of disease control with chemical IR agents that are on par with conventional pesticides may be ambitious, as we slowly move away from the philosophy of 'zero tolerance' in the control of plant pests and pathogens, the integration of chemical IR agents into IPM strategies, in which the aim is to keep pests and pathogens below the economic injury level, has merit.

We have outlined potential strategies by which the efficacy of chemical IR agents as components of IPM might be optimised (Figure 1). The efficacy of these chemicals depends

on the pathosystem in question and through experimentation, it is possible to optimise their performance. In the process of optimisation, several successful approaches have been demonstrated. The combination of chemical IR agents with plant mutualists and with other chemical IR agents have resulted in both increased protection and reduced toxicity. Similarly, chemical IR agents in combination with fungicides can reduce the required dosage of the latter. Furthermore, rational molecule design approaches hold the promise of a new and more effective generation of chemical IR agents. While in terms of breeding crops more responsive to these treatments, the phenomenon of transgenerational IR holds promise. These approaches must be based on an understanding of not only their known mechanisms of crop protection, but also the range of outcomes from experimentation with dose, environment and pathosystem combination. These are strategies that could result in considerable progress towards more robust IPM exploiting a novel range of tools to best effect and drive the development of new crop protectants designed for high efficacy in IPM application.

Figure 1: *Improving chemical IR efficacy* – Existing agents or new agents developed in rational design (Chem-IR) are tested in target pathosystems until effective agent(s) are found. The efficacy can be further improved in combination with other treatments and effective strategies can be further combined. Efficacious treatments can be tested in trans-generationally-primed plants and the cycle repeated until an optimal treatment that can be integrated in to an effective IPM strategy.

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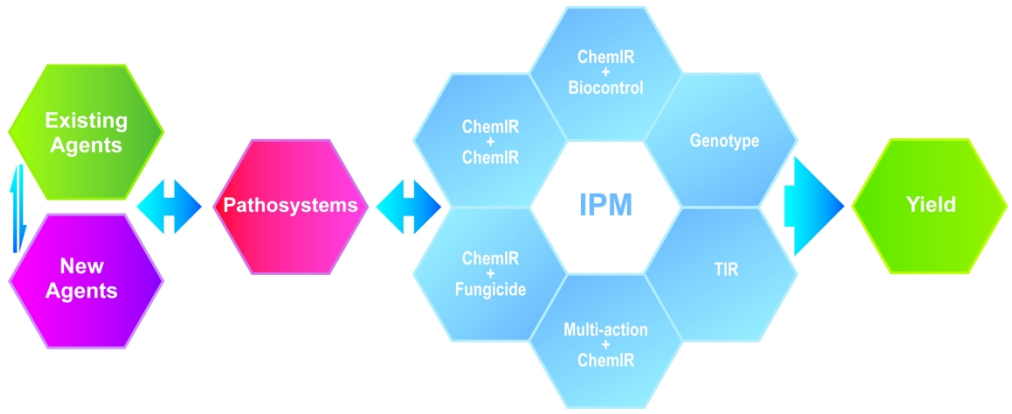

Figure 1: Improving chemical IR efficacy – Existing agents or new agents developed in rational design (Chem-IR) are tested in target pathosystems until effective agent(s) are found. The efficacy can be further improved in combination with other treatments and effective strategies can be further combined. Efficacious treatments can be tested in trans-generationally-primed plants and the cycle repeated until an optimal treatment that can be integrated in to an effective IPM strategy.

136x55mm (600 x 600 DPI)